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REFERENCE TO THE EXISTENCE OF A NON-
COAGULABLE PROTEID.

By W. H. HOWELL.

[FROM THE PHYSIOLOGICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY.]

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THE PROTEIDS OF THE BLOOD WITH ESPECIAL
REFERENCE TO THE EXISTENCE OF A
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By W. H. HOWELL.

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BLOOD serum, when made feebly acid and completely coagulated by heat, is found to contain a non-coagulable residue which may be precipitated by saturation with ammonium sulphate, by phosphotungstic acid, and by other reagents. This non-coagulable portion of the serum has been investigated by several observers, and opinions differ as to whether it is a normal constituent of the blood or an artificial product formed during the act of heat coagulation. The investigations of previous observers may be summarized briefly as follows: Chabrié seems to have been the first to call attention to this substance.¹ His method was to neutralize the serum (human) with acetic acid and heat it on the water bath until it was converted to a jelly. This jelly was cut into small pieces and extracted with boiling water. In the opalescent extract was found a proteid which he designated as albumon. It was not coagulated by heat, but was precipitated by alcohol and gave a number of the proteid reactions. Brünner² repeated Chabrié's experiments, modifying somewhat the method of preparation. He concluded that Chabrié's albumon does not pre-exist in the serum, but is split off from the serum proteids during the process of heat coagulation. As proof for this belief, he states that pure solutions of paraglobulin and serum albumin yield a similar substance when submitted to heat coagulation. This explanation seems to have been accepted generally by later writers, but it may be pointed out that the experiments given by Brünner are not entirely conclusive, since the methods used by him to prepare

¹ CHABRIÉ: *Comptes rendus de l'Académie des sciences*, 1891, cxiii.

² BRÜNNER: *Zur Kenntniss der Eiweisskörper des Blutserums*, Inaugural Dissertation, Bern, 1894.

his pure solutions of paraglobulin or serum albumin were such as might have precipitated the albumon, assuming that this proteid pre-exists in the blood. Zanetti¹ has described a similar proteid which he obtained from ox's serum. This proteid after purification by repeated precipitation by alcohol gave many of the usual proteid reactions and in addition yielded a reducing body when treated with dilute acids. Zanetti concluded that the non-coagulable residue in blood serum belongs to the group of ovomucoids. Langstein² repeated Zanetti's experiments. He found that the proteid prepared by the latter's method did not yield a reducing body in all cases, and he was inclined, therefore, to regard the reducing body, when present, as an impurity. He was convinced, however, that a non-coagulable proteid exists in the blood serum and assigned it to the group of albumoses, although no special reason for this classification was given other than the non-coagulability by heat. Bergmann and Langstein³ estimated the residual nitrogen-containing material of serum by the Kjeldahl method, after removing the coagulable proteid by heating in weakly acid reaction and in some cases after the addition of NaH_2PO_4 to $\frac{1}{2}$ per cent. The amount of the residual nitrogen varied according to the conditions from 7.67 per cent to 14.7 per cent of the total nitrogen of the blood; and examination of the substance yielding this nitrogen showed that it gave proteid reactions. They separated this non-coagulable residue into a portion consisting of primary and secondary proteoses and a portion precipitable by phosphotungstic acid. They give no conclusive proof, however, that the proteids in question belong to the group of proteoses. Kraus⁴ used a somewhat similar method. He precipitated blood plasma by heat coagulation in acid reaction, and treated the filtrate, after concentration, with $\frac{1}{10}$ its volume of saturated solution of zinc sulphate and also by saturation with sodium chloride in acid reaction. If the precipitate produced by the last reagent cleared up on heating, he concluded that all coagulable proteid had been removed. The solution was then precipitated by saturation with zinc sulphate or by phosphotungstic acid, and the amount of nitrogen contained in it was determined. The results

¹ ZANETTI: *Annali di Chimica e di Farmalogica*, No. 12, 1897; quoted from *Jahresberericht der Thier-Chemie*, 1897, xxvii, p. 32.

² LANGSTEIN: *HOFMEISTER'S Beiträge*, 1902, iii, p. 373.

³ BERGMANN and LANGSTEIN: *HOFMEISTER'S Beiträge*, 1904, vi, p. 27.

⁴ KRAUS: *Zeitschrift für experimentelle Pathologie und Therapie*, 1906, iii, p. 52.

obtained were variable, indicating that when the blood contained a total nitrogen of 2.73 to 4.47 per cent, the residual nitrogen of the non-coagulable portion varied from 0.039 to 0.146 per cent. In this as in the paper by Bergmann and Langstein no positive proof is given that the non-coagulable proteid belongs to the group of albumoses, and no proof whatever that the substance in question is not split off from the other proteids of the blood during the act of heat coagulation. So far as the residual nitrogen is concerned, it is evident that, as determined by these authors, it includes such substances as the amido acids, urea, lecithin, etc., which would be left in the serum after removing the coagulable proteid. Regarding the question of the existence of peptones or proteoses in the blood, Hofmeister, Toepfer, Embden and Knoop are quoted as having obtained positive evidence, while Neumeister, Abderhalden and Oppenheimer report negative findings.¹

As stated in an accompanying paper, the author has shown recently that when an animal is bled from the portal vein during the height of digestion, and the volume of blood obtained (250 c.c.) is dialyzed against distilled water, using a collodium membrane, no trace of peptones or proteoses can be detected in the concentrated dialysate. Since the collodium membrane is readily permeable to peptones and proteoses, and since the other diffusible constituents that exist in the portal blood, such as sugar, amido acids, ammonia salts, can be detected easily in the dialysate, it follows that if this form of proteid exists in the blood in even minute amounts it would be detectable by this means. Whether there is a proteid in the blood not coagulable by heat is, it seems to the author, a different question from whether peptones and proteoses are present. The former question is difficult of solution, but regarding the latter the evidence is so unsatisfactory that one is justified at present in denying their existence normally in the blood in amounts that can have a physiological significance.

The non-coagulable proteid in serum. — That the blood serum contains after heat coagulation a residual non-coagulable proteid is a fact beyond question, if by non-coagulable proteid is understood

¹ HOFMEISTER: *Zeitschrift für physiologische Chemie*, 1881, v, p. 127; TOEPFER: *Wiener klinische Wochenschrift*, 1902, No. 11; EMBDEN and KNOOP: *HOFMEISTER'S Beiträge*, 1903, iii, p. 120; NEUMEISTER: *Zeitschrift für Biologie*, 1888, xxiv, p. 272; ABDERHALDEN and OPPENHEIMER: *Zeitschrift für physiologische Chemie*, 1904, xlii, p. 155.

one not coagulated by heat in feebly acid reaction in the otherwise unaltered serum. The author has attempted to study this proteid using the following method. The serum (dog, beef, horse) was collected usually from a clot, although in some cases oxalate or fluoride plasma was used. The serum was diluted with once or twice its volume of a solution of sodium chloride, 0.7 per cent, and thoroughly centrifugalized. It was then heated on a water bath to 85° C. and dilute acetic acid was added, while stirring, until the reaction was permanently but feebly acid. The heavy coagulum obtained was filtered off through cotton cloth, and the serum was again heated to 85°, with the addition of more acid if necessary to maintain a feebly acid reaction, and again filtered through filter paper. The clear yellow filtrate gave no further coagulum upon heating to 85° or 100° C. when beef serum was used. Dog serum, if kept for a number of minutes at 85° to 100° C., developed an opalescence which gradually took the form of a fine precipitate. This precipitate could not be filtered off successfully in all cases. The beef serum, although it remained entirely clear or developed only a slight opalescence when boiled, also gave a similar fine precipitate on boiling if the reaction was made strongly acid with acetic acid. This precipitate brought down with it all of the pigment present, and was characterized when fresh by a delicate agreeable odor. The non-coagulable proteid left after the heat coagulation of the albumin and globulins of the serum falls, therefore, into two parts, — one which is entirely unaffected by boiling in strong acid reaction, and one which by prolonged heating to 85° to 100° C., especially in strong acid reaction, is precipitated more or less imperfectly. The qualitative reactions of these proportions were apparently the same, so that in most cases the entire proteid left in the clear filtrate from the coagulum thrown down at 85° was treated as one. The actual amount of this material and of its two parts was determined in several analyses, but the figures did not agree very closely, owing, as was discovered later, to the fact that lecithin and other substances extractable by alcohol and ether are present, and the thoroughness of this extraction alters considerably the results. The following figures, however, were obtained. The total non-coagulable proteid determined in three specimens from different animals by precipitation with ammonium sulphate and reckoned in percentages of the weight of serum used, was, respectively, 0.76, 0.85, and 0.91 per cent, an average, therefore, of 0.84 per cent. The portion coagulated at 100° C. in strong acid reaction, determined in six specimens, was

0.31, 0.31, 0.21, 0.35, 0.29, and 0.38 per cent of the serum used, giving an average of 0.31 per cent. The proteid left in the blood serum after boiling in strong acid reaction was therefore equal to about 0.5 per cent. This proteid was not a form of acid albumin, since it was not precipitated by neutralization nor by saturation with sodium chloride.

Qualitative reactions of the non-coagulable proteid. — These reactions apply to either portion of this proteid as described above.

1. It gives an excellent millon reaction.
2. It gives a blue-purple biuret reaction.
3. It gives a xanthoproteic reaction.
4. It is not precipitated by dialysis, nor by carbon dioxide after dilution.
5. It is precipitated by potassium ferrocyanide and acetic acid if the reaction is made strongly acid.
6. It is precipitated by nitric acid, the precipitate not disappearing on heating.
7. It is not precipitated by complete saturation with sodium chloride.
8. It is incompletely precipitated by saturation with magnesium sulphate.
9. It is completely precipitated by ammonium sulphate added to a saturation of 50 to 70 per cent.
10. It is precipitated by alcohol added to a concentration of 50 per cent or more.
11. It is precipitated in acid solution by phosphotungstic acid or iodine in solution in potassium iodide.
12. After dialysis and the addition of sodium, potassium, or lithium chloride it gives no precipitate on boiling. A coagulum occurs, however, under these conditions upon the addition of ammonium chloride or sulphate or the chloride of magnesium, calcium, or barium.
13. It gives always a strong reaction for phosphorus and a distinct reaction for iron. The sulphur reaction was in some cases negative.

The content in phosphorus. — It was quickly discovered that the non-coagulable proteid gives a strong reaction for phosphorus, and a number of quantitative determinations of this element were made by the ammonium molybdate method. The proteid was prepared as follows. After complete coagulation at 85° C. the filtrate was precipitated by saturation with ammonium sulphate and was purified

by three successive precipitations. The final solution was in some cases dialyzed in parchment tubes against running water and distilled water until it failed to give a reaction with barium chloride, and was then precipitated by the addition of alcohol. In other cases it was heated for two hours at a temperature of 105° to 110° C., and the precipitate then washed with boiling water until all sulphate was removed. The figures obtained by the first analyses made were very variable, ranging from 0.144 to 2.9 per cent of the weight of proteid, and it was found that this variation depended on the extent of washing of the precipitate with alcohol and ether. When the precipitate obtained by either method was thoroughly digested with boiling alcohol and then with ether, the phosphorus reaction disappeared entirely or was so small that no weighings could be made. The alcohol and ether extracts were found to contain lecithin and other related bodies, as described below. It is evident from these facts that the non-coagulable proteid does not belong to the group of nucleo-proteids or nucleo-albumins, but when precipitated carries down with it some lecithin in a form of combination such that the lecithin can be extracted by hot alcohol. Hoppe-Seyler long ago called attention to the fact that the globulins of the blood when precipitated carry down some lecithin, and the present author has found this to be the case even with the precipitate of blood serum produced by carbon dioxide. Fuld and Spiro¹ call attention to the fact that pseudoglobulin, obtained by saturating serum to 34 to 46 per cent with ammonium sulphate, always contains phosphorus. Bernert² also reports that in a milky ascitic exudate examined by him the portion of the proteid precipitated by one-half saturation with ammonium sulphate gave an abundant reaction for phosphorus, but that all of the phosphorus-containing material could be extracted by hot alcohol. Freund and Joachim³ claim to have discovered a phosphorus-containing proteid in the blood which they designate as a nucleo globulin. Since, however, they give no satisfactory evidence of obtaining from this proteid any of the characteristic dissociation products of nuclein or pseudonuclein, it remains doubtful whether the proteid they were dealing with was a nucleo-proteid or nucleo-globulin, or simply a

¹ FULD and SPIRO: *Zeitschrift für physiologische Chemie*, 1900, xxxi, p. 140.

² BERNERT: *Archiv für experimentelle Pathologie und Pharmakologie*, 1902, xlix, p. 48.

³ FREUND and JOACHIM: *Zeitschrift für physiologische Chemie*, 1902, xxxvi, p. 407.

lecithin-containing proteid from which the phosphorus-containing material might have been extracted by the action of hot alcohol. The author has prepared this so-called nucleo-globulin by the method described by Freund and Joachim, and finds that it contains lecithin and other materials extractable with alcohol and ether. After thorough extraction with boiling alcohol and with ether the residue remaining failed to yield a positive test for phosphorus.

Examination of the phosphorus extract. — The non-coagulable proteid prepared as already described was usually extracted first with warm absolute alcohol, then with a mixture of equal parts of alcohol and ether, and finally with boiling absolute alcohol. The extracts taken together were evaporated to dryness at a low temperature, and yielded a yellow waxy material very soluble in ether, but soluble with some difficulty in alcohol. This material contained much lecithin. When mixed with a drop of water and examined under the microscope, it gave beautiful myelin tubes, and when treated according to the usual method with barium hydroxide, carbon dioxide, and platinum chloride, it yielded the orange-colored crystals characteristic of cholin. Two quantitative analyses of the residue after repeated solutions in ether and evaporation at a low temperature yielded 1.25 and 1.77 per cent of phosphorus. In the first analysis the residue was weighed directly after heating for several hours at 100° , until an approximately constant weight was obtained. In the second the weight of substance used was determined by difference, that is, the non-coagulable proteid was weighed before and after extraction with the alcohol and ether. Further analyses were not made, as it seemed certain from the low percentage of phosphorus that the residue was not composed entirely of lecithin. This conclusion was verified by a qualitative examination of the residue, according to which the following separate parts could be recognized: —

I. A portion precipitable from ethereal solution by acetone, the precipitate soluble in cold alcohol. This portion contained nearly 3 per cent of phosphorus and was largely lecithin.

II. A portion precipitable from ethereal solution by acetone, but the precipitate not soluble in cold alcohol. Its ethereal solution evaporated to dryness yielded a hard waxy mass which gave an abundant reaction for phosphorus. Under the microscope it showed a granular structure, but gave no myelin figures on the addition of water.

III. A portion precipitable from its ethereal solution by the addition of alcohol. The precipitate was slightly colored and soluble in water.

The amount of this material was very small; it gave a phosphorus reaction, but failed to respond to any of the reactions for jecorin.

IV. A portion not precipitable from its ethereal solutions by acetone, soluble in cold alcohol. The residue on evaporation was oily; it gave a reaction for phosphorus, and under the microscope showed numerous crystals resembling those of fatty acids and with a melting-point at about 60° C. The residue had an acid reaction.

V. A portion not precipitable from its ethereal solutions by acetone, soluble with difficulty in cold alcohol. On evaporation it gave a hard waxy mass which gave only a faint reaction for phosphorus and no myelin figures on the addition of water.

The material available for study did not suffice for further work, but it seemed evident that in the alcohol-ether extract of the non-coagulable proteid there was no substance corresponding to jecorin, while there were present two substances at least (I and II) rich in phosphorus, one of which (I) gave the characteristic myelin figures of lecithin on the addition of water, while the other failed to give this reaction.

The fact that the non-coagulable proteid when precipitated by ammonium sulphate or alcohol yields such a large amount of lecithin and related substances suggests that it may consist of a lecitho-proteid. Several authors, notably Liebermann,¹ have contended for the existence of such a group of proteids. Osborne and Campbell,² following Hopper-Seyler, have described a compound of lecithin and nucleoproteid occurring in the yolk of the hen's egg. The assumed compound has the properties of a globulin, and the lecithin in it can be extracted by alcohol. As was stated above, the globulin, particularly the pseudoglobulin, when precipitated by salts or carbon dioxide, carries down with it some phosphorus-containing material, presumably lecithin. But the non-coagulable proteid contains a larger amount of this material. When the blood is coagulated at 80° to 85° C., the heat coagulum contains only a small amount of phosphorus in a form extractable by alcohol, while the non-coagulable proteid is relatively rich in this substance. The portion of the non-coagulable proteid that is thrown down by boiling in strong acid solution contains also much phosphorus material extractable by alcohol. The amount of lecithin or related substances contained in the non-coagu-

¹ LIEBERMANN: *Archiv für die gesammte Physiologie*, 1891, 1, pp. 25 and 55.

² OSBORNE and CAMPBELL: *Journal of the American Chemical Society*, 1900, xxii, p. 413.

lable proteid is therefore noteworthy. When this proteid is precipitated by salts (ammonium sulphate), the lecithin is carried down; when dissolved, the lecithin passes into solution with it. Alcohol splits off a part of the phosphorus-containing material very easily, but for its complete removal the prolonged action of boiling alcohol is necessary. It is possible, therefore, that the non-coagulable proteid may be a lecitho-proteid compound, whether it pre-exists in the normal blood or is formed during the process of heating. A definite compound between the proteid and the lecithin would be indicated if the amount of lecithin or phosphorus-containing material showed a constant proportion. So far as my analyses go, however, this is not the case. Results of the following character were obtained:—

- I. Weight of the non-coagulable proteid precipitated by ammonium sulphate = 0.3959 gm.
 Weight of the alcohol ether extract = 0.2480 gm.
 Percentage of phosphorus in the alcohol ether extract = 1.77 per cent.
 Percentage of phosphorus in the proteid after extraction = 0.87 per cent.
 Weight of lecithin estimated from the phosphorus . . = 0.1474 gm.
 Percentage of lecithin in the non-coagulable proteid . = 37 per cent.
- II. Weight of the non-coagulable proteid precipitated by ammonium sulphate = 0.8495 gm.
 Weight of lecithin estimated from the phosphorus in the alcohol ether extract 0.113, and in the proteid after extraction 0.092 = 0.205 gm.
 Percentage of lecithin in the non-coagulable proteid . = 24 per cent.

Although not constant, it will be seen that the amount of phosphorus-containing material in the precipitated non-coagulable proteid is very large, much greater than in the portion of the serum proteids coagulated by heat. This adherent or combined lecithin modifies the properties of this proteid, and there can be no doubt that the albumon of Chabrié and the non-coagulable proteid of the blood investigated by other authors consisted of this compound. It was not possible to determine the properties of the proteid combined with the lecithin, since the method of removing completely this latter substance, namely, by prolonged boiling with absolute alcohol, must have altered this proteid constituent. The material left after extraction with the alcohol was only partially soluble in water. The portion dissolved was precipitable by alcohol, but this precipitate in turn was only partially soluble in water. The aqueous solution thus

obtained became opalescent on boiling, and with the biuret reagent gave a redder tint than the original proteid.

The iron and sulphur contents of the non-coagulable proteid. — After removal of the phosphorus-containing bodies and other material extractable by alcohol and ether, the remaining material was found to contain sulphur and iron. A single analysis for sulphur gave for 0.7033 gm. of the proteid 0.0229 gm. of barium sulphate, or 0.45 per cent of sulphur. The content in iron was more noteworthy. The proteid material after thorough extraction with alcohol gave always a distinct reaction for iron. The possibility that this iron might have arisen from the slight amount of hæmoglobin usually present in the serum was disproved by a special experiment made with the oxalated plasma of the horse. This plasma was entirely free from hæmoglobin, as far as could be determined by its color when seen in mass, by the spectroscope and by the results of Almen's test. The non-coagulable proteid prepared in the usual way from this plasma gave an excellent iron reaction. The iron was determined quantitatively in three cases by fusing the proteid with sodium carbonate and potassium nitrate and precipitating by ammonium hydroxide. The three analyses gave, respectively, 0.19, 0.17, and 0.29 per cent of the extracted proteid. The maximum amount of iron is so small that it would seem impossible for it to form a constituent part of the proteid molecule, as is the case also with many of the iron-containing proteid compounds, such as the nucleo-albumins, found in the tissues or liquids of the body. The amount of iron is sufficient, however, to make this compound of interest in connection with the iron metabolism of the body.

The relation of the inorganic salts of the blood to the heat coagulation of the proteids. — The usual proteids of the blood serum, serum globulin and serum albumin are distinguished from each other by their reaction toward magnesium sulphate or ammonium sulphate. Moreover the two portions of the globulin usually recognized, namely, euglobulin and pseudoglobulin, are likewise differentiated by the degree of saturation with ammonium sulphate necessary for their precipitation. Every one who has worked with these proteids must have recognized the unsatisfactory nature of this classification. The portion of proteid precipitated by half-saturation with ammonium sulphate and designated as serum globulin is not a well-defined chemical substance with uniform properties, and the same may be said of the fractional portions distinguished as euglobulin and pseudo-

globulin. A solution of either one of these latter proteids submitted to dialysis exhibits a portion that precipitates and a portion that remains in solution,¹—an indication of the presence of two different substances in spite of the fact that they both come down at a certain degree of saturation with ammonium sulphate. The author has been much impressed also with the fact that the blood proteids after precipitation with ammonium sulphate exhibit quite different properties, as regards their precipitation and coagulation especially, from those shown before the action of this salt. This difference may be shown in several ways. If blood serum is dialyzed very completely, a certain amount of precipitate forms which settles to the bottom of the dialyzing flask and leaves a perfectly clear supernatant liquid that may be filtered off. If now this clear liquid is precipitated by ammonium sulphate, filtered, redissolved, and the solution again dialyzed, a further abundant precipitate comes down. The action of the ammonium sulphate has rendered a portion of the proteid susceptible of precipitation by dialysis. Again, if blood serum is precipitated by ammonium sulphate added to one-third or one-half saturation, and the portion thus isolated is redissolved, it will be found that the proteid in solution is precipitable by a less degree of saturation with ammonium sulphate than was the case with the original serum. The difference in the properties of the proteids caused by precipitation with ammonium sulphate is most clearly shown by the effect on heat coagulation. Facts of the following kind may be demonstrated. If blood serum is precipitated by one-half saturation with ammonium sulphate to remove the so-called serum globulin, and the filtrate containing the serum albumin is thoroughly dialyzed until no reaction is given with barium chloride, the solution thus obtained gives no distinct coagulum on heating. If the solution is concentrated, an opalescence, will develop on boiling, but no coagulum. Moreover, if sodium chloride, potassium chloride, or lithium chloride be added to a concentration of, say, two per cent, boiling still fails to give a coagulum; in fact, the presence of these salts may even prevent the formation of an opalescence. Addition, however, of a little ammonium sulphate or chloride, or the chlorides of magnesium, calcium, or barium will cause a flocculent precipitate when the solution is heated to 70°–75° C. On the contrary, if the precipitate of serum globulin thrown down by half-saturation with ammonium sulphate is redissolved and thoroughly dialyzed, the solution precipi-

¹ FREUND and JOACHIM: *Loc. cit.*

tates in part, and the precipitate after solution, as well as the portion not precipitated by dialysis, gives a heat coagulum at 70° – 75° C. in the presence of sodium, potassium, or lithium chlorides. These facts would seem to indicate that serum albumin is a proteid whose combinations with salts of sodium, potassium, or lithium are non-coagulable by heat, and in this respect different in a striking way from the serum globulin which coagulates readily in the presence of these salts. This conclusion is not borne out, however, by observations upon blood serum not treated with ammonium sulphate or upon blood serum completely saturated with ammonium sulphate. If, for instance, one takes normal blood serum and dialyzes it thoroughly, a certain small portion is precipitated. If this precipitate is filtered off and the filtrate is tested as to its heat coagulation in the presence of salts, no indication will be found of the existence of two proteids, one of which suffers heat coagulation in the presence of sodium, potassium, and lithium salts, and one of which remains uncoagulated. On the contrary, all of the remaining proteid that can be precipitated by heat comes down at a temperature of 85° C. or more in the presence of sodium, potassium, or lithium salts, and at a temperature of 70° – 75° C. in the presence of ammonium salts. On the other hand, if the serum is precipitated by complete saturation instead of by half-saturation with ammonium sulphate, and the precipitate, consisting of both the serum globulin and serum albumin, is redissolved and thoroughly dialyzed, a certain portion of the proteids will be precipitated, the precipitate coming down in two parts,—one, the larger portion, appearing early in the dialysis, and one later, after the salts have been removed more completely. If these precipitates are filtered off and the clear, thoroughly dialyzed solution is heated after the addition of sodium or potassium chloride, all the proteid capable of heat coagulation comes down at 80° C. or below. It would seem from these results that so-called serum albumin left after half-saturation with ammonium sulphate is incoagulable by heat if no salts are present except those of sodium or potassium, but if first precipitated by ammonium sulphate, it is rendered coagulable in the presence of sodium and potassium salts also. It would seem evident from these facts that once the proteid of the blood serum or any portion of it is precipitated by ammonium sulphate its properties become altered; one may say in general that its solubility is decreased or its tendency to precipitation is increased. It is possible that this change may be due to the fact that the precipitate

with ammonium sulphate is a compound of the proteid and this salt, and that when redissolved it goes into solution in this form as a proteid compound different from that formerly existing in the blood. Moreover, when a portion of the blood proteid (the globulin) is precipitated by ammonium sulphate, the portion left behind (the albumin) is also altered, as is shown by the difference in reaction toward heat coagulation in the presence of sodium and potassium salts, between serum dialyzed before and after half saturation with ammonium sulphate. The serum albumin in the latter case is not coagulable by heat in the presence of sodium or potassium salts alone, while in the former case it is completely coagulable in the presence of these salts, although with some difficulty. It would seem that the ammonium sulphate in throwing down a portion of the proteid (serum globulin) had rendered it more easily coagulable, but had left the albumin in a less readily coagulable form. All of these facts tend to throw suspicion upon our present system of classification of blood proteids so far as it is based upon the separation by saturation with the sulphates of magnesium, ammonium, or zinc. Precipitation by these salts alters the properties of the portion precipitated as well as of the portion left in solution. This fact, taken with the further fact that the portions of proteid separated by one-third or one-half saturation with ammonium or zinc sulphate fail to exhibit uniform reactions, would indicate that the method of salting out forms artificial lines of demarcation between the different proteids of the blood. To define a globulin as a proteid precipitated by half saturation with ammonium sulphate or by saturation with magnesium sulphate is unsatisfactory, since the portion left behind as serum albumin, if itself precipitated by further saturation with ammonium sulphate, may exhibit the properties of a globulin as tested by this reaction. The group definition that we give to the globulins is that they are insoluble in water free from neutral salts, and in accordance with this definition it would seem to be more appropriate to restrict the term serum globulin to that portion of the serum proteid which is precipitated upon complete dialysis. The proteid so precipitated seems to contain no lecithin or other phosphorus-containing body. When dissolved in dilute solutions of sodium chloride, it coagulates at 68° to 70° C, the precipitation being complete and precise. The proteid left in solution after the dialysis is, according to the usual nomenclature, a mixture of globulins and albumin, but the so-called globulin remains in solution no matter how complete the dialysis

may be. If, however, the remaining proteid is precipitated by ammonium sulphate, redissolved, and again dialyzed, a very large proportion will be precipitated. Evidently this portion is altered in some way by the action of the ammonium sulphate; a proteid that under its normal conditions acted like an albumin now gives the distinctive reaction of a globulin. The proportion of the proteid precipitable by dialysis seems to be greater when the serum is entirely saturated with ammonium sulphate than when it is half saturated with this salt, — a fact which would indicate that when once the proteid or any portion of it has come down in combination with ammonium sulphate, the presence of this salt in combination renders it less soluble and more easily precipitated by dialysis or heat. It would seem to be more consistent to designate as globulin only that portion of the serum proteid which is precipitated by dialysis when the unchanged serum is submitted to this process, and to call the remainder serum albumin, since it remains in solution in spite of the removal of all diffusible salts. Since, moreover, this portion forms a combination of some kind with lecithin or a lecithin-containing complex upon which its properties depend, it might be more appropriate to speak of it as a lecitho-albumin compound. However precipitated, some phosphorus-containing material may be extracted from the precipitate, whereas the proteid thrown down from the original serum by dialysis alone is free from this material.

Does a non-coagulable proteid exist in the blood? — From the facts described in the foregoing pages it is evident that the precipitability of the proteids, and especially their property of heat coagulation, depend upon the character and quantity of the salts present. In the presence of ammonium sulphate and a slightly acid reaction all of the proteid comes down upon heating. But in serum containing only its normal salts there is a portion not coagulable by heat even when the reaction is distinctly acid. Some of the properties of this portion of the proteid have been described, and it is evident from these properties that it does not belong to the group of proteoses and peptones. The important question arises as to whether this proteid exists normally in the blood or is an artificial product split off during the process of heating. This question is difficult or impossible to answer in a satisfactory way. As isolated by the process of heating, it is a proteid differing from both the serum globulin and the albumin as ordinarily described. It is characterized by its large content of phosphorus and iron; it differs from the serum albumin in being

more readily precipitated by ammonium sulphate, and in giving, when thoroughly dialyzed, a blue-purple reaction with the biuret test instead of a reddish purple; it differs from the serum globulin, on the other hand, in not being coagulated by heat after complete dialysis when salts of sodium, potassium, or lithium are added, resembling in this regard the so-called albumin. If, following the usual method of separation, solutions of euglobulin and pseudoglobulin are prepared by fractional precipitation of the serum with ammonium sulphate, and are then completely dialyzed to remove the ammonium sulphate, it will be found that on the addition of sodium or potassium chloride to the dialyzed solution the euglobulin is practically completely precipitated by heating in acid reaction, while the pseudoglobulin is incompletely precipitated, and the portion left in solution resembles the non-coagulable proteid left after heating the whole serum. Serum albumin prepared by repeated crystallization or by precipitating the serum with acetic acid after half saturation with ammonium sulphate is, as stated above, not coagulated at all by heating in the presence of sodium or potassium salts, but in the presence of a small amount of ammonium sulphate or chloride is practically completely precipitated upon heating. If therefore the non-coagulable proteid pre-exists in the serum, it is thrown down, in part at least, with the serum globulin as usually prepared. The author, however, while unable to reach any perfectly satisfactory conclusion in this matter, has been convinced that this non-coagulable proteid is in reality an artificial product split off from the complex material present in the normal serum. This conclusion is based partly upon the inconstancy in the amount and the reactions of this material and partly upon the following consideration: When the whole serum is dialyzed, a portion of the proteid is precipitated, the true globulin. If to the balance of the serum one adds sodium chloride or potassium chloride to the strength of 1 to 2 per cent, and boils, practically all of the proteid is precipitated after one or two heatings. Subsequent addition of ammonium or barium chloride followed by heating causes no further precipitate. If the non-coagulable proteid were present, it should be left in solution, to be thrown down upon subsequent heating with barium or ammonium chloride, since its solutions are incoagulable in the presence of sodium or potassium salts alone. While this argument is not conclusive, none of the facts that may be urged in favor of the pre-existence of this proteid is free from the objection that it may be split off during the process of heating in acid solutions.

SUMMARY.

1. After complete coagulation of blood serum at 80°–85° C. in feebly acid reaction (acetic acid), there remains in solution a proteid, Chabrié's albumon, which is not coagulated by boiling, but which may be incompletely precipitated by prolonged boiling, especially if the reaction is made strongly acid.

2. This non-coagulable proteid is not a peptone or proteose. When precipitated by nitric acid, the precipitate does not clear up on warming: it gives a blue-purple biuret reaction; it is not readily diffusible, and in the presence of salts of ammonia, barium, calcium, or magnesium is coagulated by heating. When isolated from its solutions by precipitation with ammonium sulphate, added to one-half saturation, or by alcohol, it yields considerable phosphorus-containing material (lecithin), which may be removed completely by extraction with boiling alcohol. It contains also a distinct amount of iron.

3. Blood serum from the fed or starved animal when submitted to dialysis in collodium tubes gives no indication of the presence of a perceptible amount of peptone or proteose.

4. Serum albumin isolated in the usual way by half saturation of the serum with ammonium sulphate followed by complete dialysis, or precipitated from its solutions by acetic acid after the removal of the globulins and then redissolved and dialyzed, shows the peculiarity that its solutions when heated in the presence of sodium, potassium, or lithium salts give no heat coagulum. Addition of small amounts of ammonium sulphate or chloride, or barium, calcium, or magnesium chloride, causes the formation of a coagulum on heating. Solutions of the globulins after precipitation by ammonium sulphate, followed by complete dialysis of their solutions, are coagulated by heating in the presence of salts of sodium, potassium, and lithium, as well as those of ammonia, barium, calcium, and magnesium.

5. The separation of the proteids of the serum into several globulins and albumin by means of fractional precipitation with ammonium sulphate is unsatisfactory, inasmuch as the products isolated do not show uniform properties. Evidence is given that after a proteid has been salted out by ammonium sulphate its properties are changed in the direction of a greater susceptibility to precipitation by dialysis or heating. The reactions of the proteids, particularly the heat coagulation, must be considered with reference to the kind as well as the amount of salts present in the solution.

6. It is suggested that the term serum globulin be reserved for the proteid precipitated from normal serum or plasma by complete dialysis. This proteid shows a uniform and precise temperature of heat coagulation in solutions containing sodium chloride. The portion of the serum proteid not precipitated by dialysis shows the essential properties of an albumin modified by combination with lecithin or a lecithin-containing complex.

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